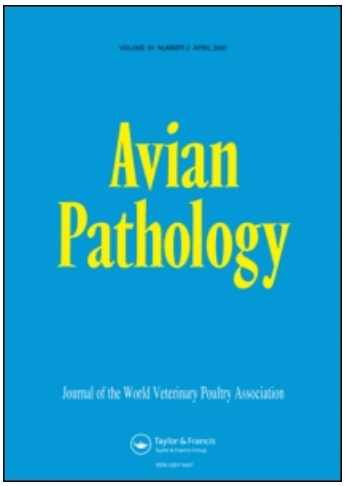

Avian Pathology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713405810

Unusual pathology of canary poxvirus infection associated with high mortality in young and adult breeder canaries (Serinus canaria)

H. L. Shivaprasad [a]; T. Kim [b]; D. Tripathy [b]; P. R. Woolcock [a]; F. Uzal [c]
[a] California Animal Health and Food Safety Laboratory System, Fresno Branch [b] Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana, IL, USA [c] California Animal Health and Food Safety Laboratory System, San Bernardino Branch, University of California, Davis, USA

Online Publication Date: 01 August 2009

To cite this Article Shivaprasad, H. L., Kim, T., Tripathy, D., Woolcock, P. R. and Uzal, F.(2009)'Unusual pathology of canary poxvirus infection associated with high mortality in young and adult breeder canaries (Serinus canaria)',Avian Pathology,38:4,311 — 316
To link to this Article: DOI: 10.1080/03079450903061643
URL: http://dx.doi.org/10.1080/03079450903061643

Avian Pathology (August 2009) **38(4),** 311–316

Taylor & Francis
Taylor & Francis Group

Unusual pathology of canary poxvirus infection associated with high mortality in young and adult breeder canaries (*Serinus canaria*)

H. L. Shivaprasad[1]*, T. Kim[2]†, D. Tripathy[2], P. R. Woolcock[1] and F. Uzal[3]

[1]*California Animal Health and Food Safety Laboratory System, Fresno Branch,* [2]*Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana IL 61802, USA, and* [3]*California Animal Health and Food Safety Laboratory System, San Bernardino Branch, University of California, Davis, USA*

Mortality in excess of 65% occurred in a flock of 450 canaries (*Serinus canaria*). Clinical signs in the canaries included severe respiratory distress, loss of feathers and/or scaly skin on the head, neck and back, anorexia, loss of weight and fluffed-up appearance of several days duration before death. Gross pathology in most of the canaries included thickened eye lids and small scab-like nodules on the skin of the head and neck, enlarged thymus, mild to severe consolidation of lungs and exudate in the sinuses and trachea. A few birds also had thickened air sacs and enlarged and pale spleens. Microscopically unusual lesions included severe epithelial proliferation and hypertrophy and mononuclear inflammatory cells containing eosinophilic intracytoplasmic inclusion bodies of poxvirus in the thymus, bursa of Fabricius, spleen, bone marrow, air sac, peritoneum, external and middle ears, and lachrymal gland. Similar inclusion bodies associated with inflammation were also seen in the epidermis, dermis, feather follicles, conjunctivae, sinuses, turbinates, choana, oral mucosa including tongue, oesophagus, larynx, trachea, syrinx and bronchi and parabronchi of lungs. Some of the birds also had concurrent bacterial, mycotic and polyomavirus infections. Poxvirus was isolated from lungs and skin in chicken embryo liver cells and confirmed as avian poxvirus by polymerase chain reaction.

Introduction

Avian pox is a slow-spreading viral disease of many species of birds caused by viruses of the family *Poxviridae*, known usually as poxviruses (Gerlach, 1994; Bolte *et al*., 1999; Tripathy & Reed, 2008). It has been reported in 232 species of birds of 23 orders (Bolte *et al*., 1999; Tripathy & Reed, 2008).

Poxviruses are large, oval or brick-shaped enveloped DNA viruses and measure about 330 nm × 280 nm × 200 nm (Bolte *et al*., 1999; Tripathy & Reed, 2008). Avipoxvirus belongs to the *Chordopoxvirinae* subfamily and contains 10 species and two additional tentative species (Tripathy & Reed, 2008). The canary poxvirus (CAPV) is one of these species. CAPV is responsible for canary pox, which is a highly infectious disease that may produce up to 100% mortality in affected aviaries and may also occur in a latent form with the virus being shed from lesions for up to 13 months (Johnson & Castro 1986; Bolte *et al*., 1999; Tripathy & Reed, 2008). Secondary pathogens such as bacteria and fungi can contribute significantly to the mortality (Johnson & Castro, 1986). The disease is usually transmitted through a break in the skin, most commonly caused by biting vectors, such as mosquitoes. But in canaries vectors may not be necessary, as the virus can gain entrance through the respiratory route and cause pneumonia. Canary pox can also cause serious disease in different species of passerines such as sparrows and finches (Giddens *et al*., 1971; Donnely & Crane, 1983; Theil *et al*., 2005).

The disease in canaries is primarily septicaemic with anorexia, lethargy, ruffled plumage, respiratory distress and death occurring within 3 to 18 days of infection (Burnet & Bernard, 1933; Giddens *et al*., 1971; Donnely & Crane, 1983; Johnson & Castro, 1986; Gerlach, 1994). Small crusty lesions around the beak and eye lids can also occur. The most common pathological change is proliferative bronchopneumonia with many bronchial epithelial cells containing eosinophilic intracytoplasmic inclusion bodies (Burnet & Bernard, 1933; Giddens *et al*., 1971; Donnely & Crane; 1983, Johnson & Castro, 1986; Gerlach, 1994). Other uncommon lesions such as epidermitis involving the skin on the various parts of the body, rhinitis and occasionally oesophagitis, pleuritis, airsacculitis and peritonitis have been reported (Giddens *et al*., 1971; Donnely & Crane, 1983; Gerlach, 1994). The present paper describes an outbreak of poxvirus infection associated with high mortality in Color-breeder canaries characterized by lesions that have not been described before.

*To whom correspondence should be addressed. Tel. +1 559 498 7740. Fax: +1 559 485 8097. E-mail: hlshivaprasad@ucdavis.edu
†Current Address: Avian Disease and Oncology Laboratory, USDA-ARS, 3606 East Mount Hope Road, East Lansing, MI 48823, USA.
Received 24 February 2009
ISSN 0307-9457 (print)/ISSN 1465-3338 (online)/09/040311-06 © 2009 Houghton Trust Ltd
DOI: 10.1080/03079450903061643

Materials and Methods

Case history. The outbreak took place in the months of March and April. The canaries (*Serinus canaria*) affected were from a large breeding colony containing approximately 450 canaries of different Color varieties. There were 175 adults and 275 young canaries in the aviary. The facility was approximately 20 feet × 10 feet and contained large flight cages and nest boxes for breeders. Birds were provided a diet consisting of canary mix of seeds, vitamins and minerals. Fresh water was available *ad libitum*. Fourteen canaries were introduced in to the aviary without prior quarantine a few weeks before the problem started. Clinical signs described for the canaries prior to death were severe dyspnoea, anorexia, weakness, rapid loss of weight, fluffed-up appearance, loss of feathers and/or scaly skin on the head, neck and back, and weakness of several days duration. The total number of birds that died and/or were culled over a 1-month period was 255 (including 170 young canaries and 85 adult canaries). The remaining birds were vaccinated against canary pox with Poximune (Biomune, Lenexa, Kansas, USA) in the wing web.

Pathology. Six live and two dead canaries between 4 and 8 weeks of age, and one live male and one dead female adult canaries were submitted for postmortem examination to the San Bernardino and Fresno branches of the California Animal Health and Food Safety Laboratory System. The live birds submitted to the laboratory were euthanized with carbon dioxide, and blood was collected immediately after death. All birds were weighed and necropsies were performed. Sections of brain, spinal cord, bursa of Fabricius and thymus, if present, bones, ears, eyes, conjunctiva, heart, liver, spleen, kidneys, thyroids, adrenals, pancreas, testes, ovary, oviduct, skin, skeletal muscle, and sections of the entire digestive tract (oesophagus, crop, proventriculus, gizzard, intestine) and sites in the respiratory tract (nasal passages, air sac, larynx, trachea, syrinx, and lungs) were collected, fixed in 10% buffered neutral formalin, embedded in paraffin, sectioned at 4 μm, stained with haematoxylin and eosin (H & E), and examined by bright-field microscopy. Selected tissues were also stained with MacCallum Goodpasture, Brown–Brenn Gram methods and Periodic acid Schiff according to the standard methods.

Electron microscopy. Portions of formalin-fixed skin and lung from two young canaries were placed in half-strength Karnovsky's fixative followed by aldehyde fixative and processed for thin-section electron microscopy following standard methods. Briefly, after aldehyde fixation, tissues were washed in 0.2 M sodium cacodylate, post-fixed in 2% osmium tetroxide reduced with 2.5% potassium ferrocyanide, dehydrated in ethanol and infiltrated and embedded in Spurr's epoxy resin formulation. Thin sections were cut and mounted on 150-mesh copper grids, stained briefly with 6% methanolic uranyl acetate, post-stained in Reynold's lead citrate and observed in a LEO 906 transmission electron microscope at 60 kV accelerating voltage.

***In situ* hybridization for circovirus and polyomavirus.** Sections containing the thymus, bursa of Fabricius, spleen, liver, heart, kidney, and skin, from four young canaries were submitted to the DNA IN SITU Hybridization Laboratory, University of Georgia, Athens, Georgia, USA for detecting polyomavirus (VP-1 probe) and circovirus (generic probe) nucleic acid by the *in situ* hybridization technique (Garcia *et al*., 1994; Woods & Latimer, 2000). The VP-1 probe had been used to detect nucleic acid of polyomavirus in psittacines and passerines (Garcia *et al*., 1994). The generic probe of circovirus had been used to detect nucleic acid of circoviruses in psittacines, pigeons and finches (Woods & Latimer, 2000).

Serology. Sera from five birds were tested by standard methods for antibodies to avian Paramyxovirus type I by haemagglutination inhibition test, using the reagents from the National Veterinary Services Laboratory (Ames, Iowa, USA).

Bacteriology. Swabs from the liver, trachea and intestine were plated on 5% sheep blood and MacConkey agars (Remel, Lenexa, Kansas, USA) and incubated at 37°C with 5% CO_2 for a minimum of 48 h. The intestinal contents were also selectively enriched in selenite enrichment broth at 42°C for 18 to 24 h. Following enrichment, selenite broth was plated on brilliant green and XLT4 agars (Remel) and incubated at 37°C for an additional 24 h. Swabs of trachea and conjunctiva were also plated onto modified Frey's agar (Frey) and inoculated into Frey's broth and incubated at 37°C in 7.5% carbon dioxide and 98% humidity for the isolation of *Mycoplasma* spp. The plates were observed for 14 days for typical mycoplasma colonies. Broth cultures were plated on to Frey's agar after 7 days of incubation and the plates were observed for 7 days.

In addition, all birds were examined for *Chlamydophila psittaci* using impression smears prepared from the liver, spleen and conjunctiva with a commercially available immunofluorescence reagent (Bartel's, Wicklow, Ireland) according to the manufacturer's directions.

Virus isolation. Pools of skin and lung tissues from three canaries were mixed with buffered saline containing antibiotics, triturated with sterile silica with a pestle and mortar, and clarified by centrifugation at 1780 × *g* for 10 min. An aliquot of the supernatant was passed through a 0.45 μm membrane filter. The filtrate was inoculated onto monolayers of primary chicken embryo liver cells and incubated at 37°C in an atmosphere containing 5% carbon dioxide for 5 days. The cells were harvested, frozen and thawed three times and centrifuged at 1780 × *g* for 10 min. The supernatant was then inoculated on to primary chicken embryo liver cells and reincubated again; after 4 to 5 days, a cytopathic effect was apparent. The cell culture fluid was harvested and submitted for examination by negative-stain electron microscopy.

Negative stain electron microscopy. Harvested cell culture fluid was clarified by centrifugation at 1780 × *g* and then centrifuged at 140 000 × g for 75 min to pellet the virus. Pellets were resuspended in 0.3 to 1.0 ml deionized water and 2 to 3 μl was mixed with 100 to 200 μl of 0.8% phosphotungstic acid; a drop was then placed on to a 200-mesh formvar-coated grid for 3 to 5 min. The unabsorbed material was wicked away with filter paper. Grids were examined on a Zeiss EM 10A electron microscope and typical poxvirus particles were observed.

Isolation of viral DNA. Viral DNA was isolated from cell culture fluid using a slight modification of a previously described technique (Schnitzlein *et al*., 1988) with modification. Briefly, virus-infected cell monolayers were scraped and the cells were pelleted by centrifugation at 1500 × *g* for 10 min at 4°C, washed with isotonic buffer (10 mM Tris, pH 8.0, 150 mM NaCl, 5 mM ethylenediamine tetraacetic acid) and lysed in 10 ml hypotonic buffer (10 mM Tris, pH 8.0, 150 mM KCl, 5 mM ethylenediamine tetraacetic acid) containing 0.025% β-mercaptoethanol and 0.1% triton X-100. After removal of cell nuclei by centrifugation at 1000 × *g* for 5 min at 4°C, the viral cores were pelleted by centrifugation at 11 000 × *g* for 90 min at 4°C. Viral DNA was extracted from the pelleted cores by DNAzol® reagent (Invitrogen, Maryland, USA) according to the manufacturer's instructions.

PCR amplification of avian poxvirus and reticuloendotheliosis virus sequences. To confirm the virus isolated from canaries as avian poxvirus, polymerase chain reaction (PCR) amplifications were conducted using fowlpoxvirus (FWPV) and reticuloendotheliosis virus (REV) sequences specific primer as described earlier (Table 1) (Kim *et al.*, 2003). Degenerate primer set for flanking REV long-terminal repeat (LTR) remnant sequences also was used (Kim *et al*., 2003). The PCR mixture and conditions were as described earlier (Kim & Tripathy, 2001). The amplicons were analysed by gel electrophoresis using 0.8% agarose.

Comparison of viral DNA restriction fragment length polymorphism. In order to examine the relationship between isolated CAPV and FWPV, viral DNAs that isolated as above digested *Hin*dIII (GibcoBRL) and restriction fragments were analysed by gel electrophoresis.

Results

The seven live canaries (*S. canaria*) were in fair to good flesh and weighed between 17 and 24 g. Three canaries that were submitted dead were in mild to moderate state

Table 1. *PCR primer sequences for amplification of avian poxvirus and REV sequences*

Specific for	Sequence
FPV envelope	Forward: 5′-CATACATTACTCTTAATTCGTTTC-3′
	Reverse: 5′-TTGTAACTGTTCTATTAGTGCC-3′
REV 5′ LTR sequence	Forward: 5′-CATACTGGAGCCAATGGTT-3′
	Reverse: 5′-AATGTTGTAGCGAAGTACT-3′
REV envelope	Forward: 5′-GAAGCAGACAATAGGACTGG-3′
	Reverse: 5′-CCTCGAGGTCAAATCATTGACCTAGG-3′
Flanking sequences of REV integration	Forward: 5′-YGGAGAYAGRSAAATATCAGA-3′
	Reverse: 5′-ATARKAATCACMMGWATATACC-3′

of postmortem decomposition at the time of necropsy. Grossly, seven canaries (six young and one adult) had thickened skin and lacked feathers on the head including eyelids and neck and back. Five young canaries and one of the adult canaries had consolidation of the cranioventral lobes of both lungs around the primary bronchi and a moderate amount of frothy exudate in the trachea. The female adult bird had severe consolidation of the lungs and a large amount of grey exudate in the lumen of the larynx and trachea. Yellow frothy fluid was present in the infraorbital sinuses and nasal passages in several other birds. The two adult birds had thickened abdominal air sacs and five of the young birds had enlarged anterior thymic lobes. Two young birds had mild to moderately enlarged pale spleens.

Microscopically, six out of eight young canaries and one out of two adult canaries (*S. canaria*) had lesions of poxvirus. In five out of eight young canaries the normal architecture of the thymuses was completely effaced due to the presence of hyperplastic and hypertrophied mononuclear cells (Figure 1), most of which contained poxvirus inclusions in their cytoplasm. The inclusion bodies were deeply eosinophilic, fairly large but of different sizes, and many contained one or two vacuoles of various sizes (Figure 2). Some of the larger inclusion bodies displaced the nucleus to one side of the cell. Some of the thymuses affected by poxvirus were difficult to recognize as thymus. In the thymuses of three birds there were occasional epitheliod cells that contained polyomavirus-like inclusions in their nucleus. The bursas of Fabricius had nodules corresponding to the follicles and were composed of similar cells containing poxvirus inclusions as in the thymus (Figure 3). The spleens in three canaries had increased number of mononuclear phagocytic cells, some of which contained similar intracytoplasmic inclusions (Figure 4). In the bone marrow of the skull interspersed between osteocytes there were large number of mononuclear cells, many of which contained intracytoplasmic inclusion bodies (Figure 5a). Also in the bone marrow of the skull there were occasionally cords of hyperplastic and hypertrophied cells that contained similar intracytoplasmic inclusions (Figure 5b). Similar cords with intracytoplasmic inclusions were also observed in the middle ears of a few birds.

Skin over the eyelids, head, neck and external ears and a few feather follicles had multifocal acanthosis, and occasionally forming large nodules, due to hyperplasia and hypertrophy of epithelial cells (Figure 6). There were many vesicles within the epidermis with occasional cleft formations and ulcerations (Figure 6). There was intense mononulcear cell inflammation in the underlying dermis, some of which contained eosinophilic intracytoplasmic inclusion bodies (Figure 7). The epidermis in most birds especially around the head and neck not only had acanthosis but also hyperkeratosis, sometimes associated with small yeast forms of Candida sp.—most probably *Candida globrata.* Some of the feather follicles also contained numerous such yeast forms.

The epithelium of the air sacs and the peritoneum and ducts of the lachrymal gland, the mucosa of the conjunctiva, sinuses, turbinates, larynx, trachea syrinx, bronchi and parabronchi were moderate to severely thickened due to proliferation of epithelial cells and contained poxvirus inclusions accompanied by lymphoplasmacytic inflammation. There was severe fibrinosuppurative inflammation in the trachea and bronchi and parabronchi of lungs associated with coccoid bacteria.

In one bird there were numerous mononuclear cells around the bronchi and parabronchi of the lungs that contained karyomegalic faintly staining basophilic intranuclear inclusion bodies consistent with polyomavirus. Three birds had similar intranuclear polyomavirus inclusions in the mononuclear cells of the thymus and spleen.

Other changes in the canaries included mild to severe lymphoplasmacytic inflammation of the mucosa in the proventriculus associated with a few to large numbers of fungi *Macrorhabdus ornithogaster* (previously called megabacteria) in seven birds, mild infiltration of protozoa of *Trichomonas* spp. in the mucosa of the oesophagus in six birds, and mild periportal lymphoplasmacytic inflammation of the liver and heart and the interstitium of the lungs in a few birds. There were a few coccidia in different stages of development in the intestine of a few canaries. No significant histological lesions were seen in the other tissues examined.

Electron microscopy. Electron microscopic examination of the skin and lungs of two young canaries revealed numerous viral particles in the cytoplasm of epithelial and mononuclear cells consistent with poxviruses (Figure 8a,b). These virions were 300 to 450 nm in diameter, brick-shaped, with a nucleocapsid and an intermediate coat and envelope interspersed between the scattered surface tubules (Figure 8b). In addition, within the nucleus of the mononuclear inflammatory cells of the lung there were icosahedral virus particles measuring 40 to 45 nm in diameter arranged loosely consistent with polyomavirus.

***In situ* hybridization for circovirus and polyomavirus.** All of the tissues examined from four canaries for circovirus and polyomavirus nucleic acid by the *in situ* hybridization technique were negative.

Bacteriology. *Staphylococcus aureus* was isolated from the lung of one bird and *Corynebacterium* spp. was isolated from the trachea, conjunctiva and infraorbital sinus of three canaries. No other bacterial pathogens including *Salmonella* spp. and mycoplasma were isolated from any canaries. All of the birds tested for *Chlamydophila psittaci* were negative.

Serology. The four birds were tested for avian paramyxovirus—one was negative.

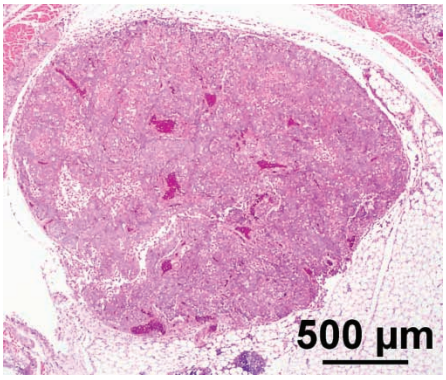

Figure 1. *Thymus with complete loss of architecture and replacement by mononuclear inflammatory cells due to poxvirus infection. H & E.*

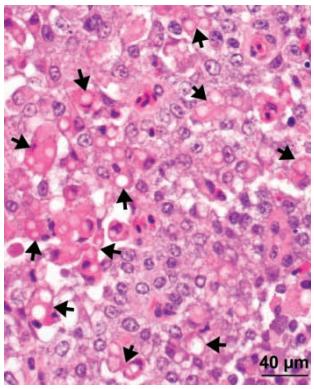

Figure 2. *Higher magnification of Figure 1 showing mononuclear inflammatory cells containing numerous eosinophilic intracytoplasmic inclusions with vacuoles (arrows). H & E.*

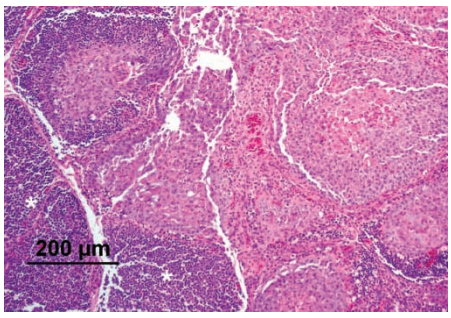

Figure 3. *Bursa of Fabricius with complete replacement of most follicles with hypertrophied and hyperplastic mononuclear inflammatory cells due to poxvirus infection. Note normal follicles (*). H & E.*

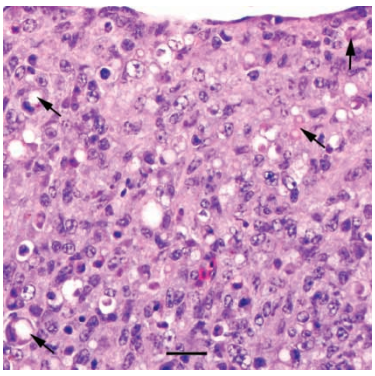

Figure 4. *Spleen with a few mononuclear inflammatory cells containing intracytoplasmic inclusion bodies of poxvirus, many with vacuoles (arrows). H & E. Bar = 40 µm.*

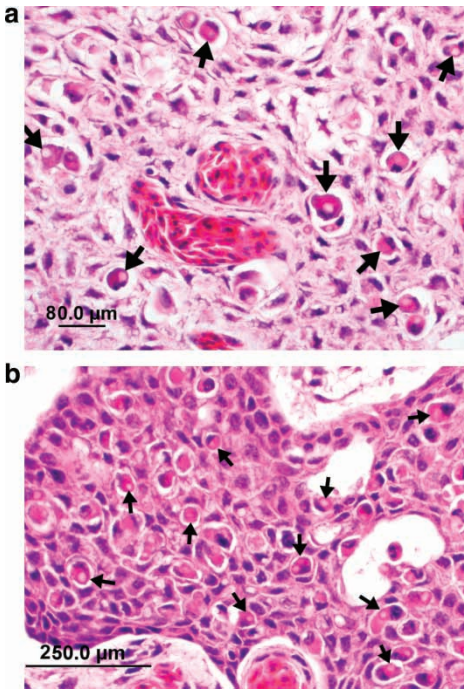

Figure 5. *Bone marrow with mononuclear cells containing intracytoplasmic inclusion bodies of poxvirus (arrows) interspersed between (5a) osteocytes and (5b) chords of epithelial cells. H & E.*

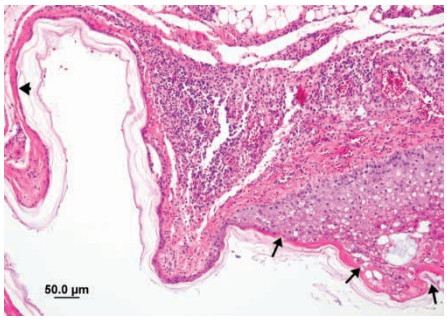

Figure 6. *Epidermis of external ear with severe acanthosis (arrows) due to hyperplasia and hypertrophy of epithelial cells due to poxvirus (arrowhead, tympanic membrane). H & E.*

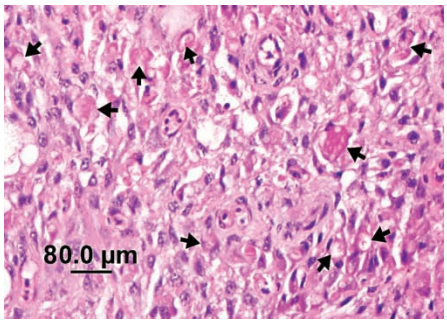

Figure 7. *Dermis from the head of a canary with severe inflammation; some of the mononuclear inflammatory cells contain eosinophilic intracytoplasmic inclusion bodies of poxvirus (arrows). H & E.*

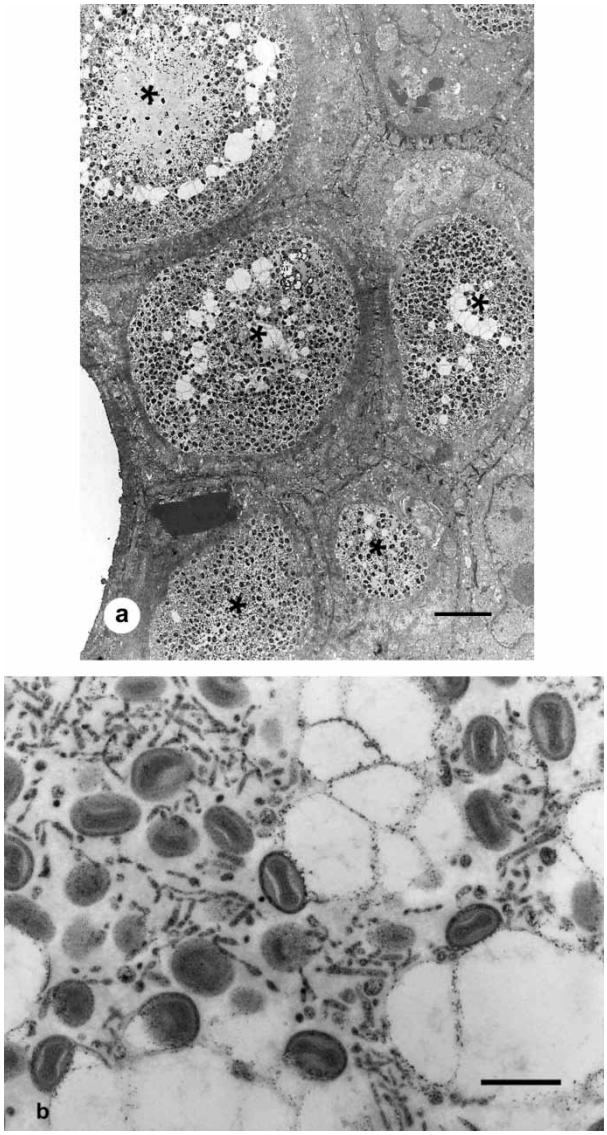

Figure 8. *Electron micrographs of the skin showing a large number of poxviruses in the cytoplasm of hypertrophied epithelial cells (*) (a) (Bar = 1.5 mm) containing typical poxviruses, (b) (Bar = 500 μm). Uranyl acetate and lead citrate.*

Virology. Avian pox was isolated from the lungs and skin of two of the three canaries subjected to virus isolation.

PCR amplification of FWPV and REV specific sequences in CAPV genome. Specific FWPV sequences were not amplified in the genome of CAPV isolates. Also, none of the REV sequences (envelope and LTR remnant sequences) were amplified in their genome. Only flanking sequences of REV integration were amplified using degenerate primers in CAPV (652 base pairs), and showed a smaller product than that of FWPV (943 base pairs). Differences in amplicon size between FWPV and CAPV was caused by REV LTR remnant sequences in the genome of FWPV (Kim *et al*., 2003).

Comparison of restriction enzyme digestion patterns of CAPV. To determine the relationship of CAPV to FWPV, each CAPV isolate viral DNA was digested with *Hin*dIII, and generated DNA fragments were compared. Overall, the DNA fragment of two CAPV isolates showed similarity to each other; however, these are quite different from the restriction digestion fragment of FWPV. No DNA fragments were shown in FWPV between 10 kb and 8 kb. Distinct fragments of approximately 12 kb, 9.4 kb, 6.2 kb and 5.5 kb were shown in CAPV isolates.

Discussion

Clinical signs of respiratory distress with lesions in the upper and lower respiratory tract associated with characteristic intracytoplasmic inclusions of poxvirus were observed in most canaries of this study. In this outbreak, poxvirus infection was probably the primary infection that, in combination with other pathogens (i.e. bacteria and fungi such as megabacteria), resulted in high mortality. This has been documented in similar epornitics of canary pox (Donnely & Crane, 1983; Johnson & Castro, 1986; Gerlach, 1994). In addition, some of the birds had what appeared to be concurrent polyomavirus infection, which may have also contributed to the clinical signs and mortality in the canaries. Avian polyomaviruses are DNA viruses that cause a multisystemic disease called Budgerigar Fledgling disease with high mortality in psittacines and occasionally in passerines such as Finches and Seedcrackers (*Pyrenestis* sp.) and blue bills (*Spermophaga haematina*) (Garcia *et al*., 1994; Wittig *et al*., 2007). However, polyomavirus infection in canaries has not been documented previously. In psittacines the disease is characterized by karyomegalic cells with faintly staining bluish amphophilic intranuclear inclusions in various organs. Similar inclusions were seen in the lungs, spleen and thymus of the three young canaries in the present study. In addition, thin-section electron microscopy of lungs in these birds revealed the presence of virus particles consistent with the size and morphology of polyomavirus. *In situ* hybridization for polyomavirus was negative, suggesting either that the polyomavirus identified in canaries is a different virus from psittacines or that the VP-1 probe (Garcia *et al*., 1994) used in this study does not identify the polyomavirus infecting canaries.

The most common microscopic lesions associated with poxvirus in canaries are in the respiratory and integumentary (epidermis) systems, although other systems can also be affected. However, lesions in the thymus, bursa of Fabricius, spleen, dermis, external and middle ears, bone marrow and lachrymal gland that were present in the canaries in the present study have not been described before. It is probable that this may represent severe viraemic (septicaemic) form of the disease. Although it is not known why these organs were affected in our canaries, it is possible that the poxvirus infecting the canaries was more virulent than other previously reported strains. Concurrent polyomavirus infection might have played a role in predisposing at least some of the canaries to such lesions. Also, polyomaviruses are frequently opportunistic pathogens that turn from latency to productive infection as a consequence of immunosuppression. Therefore, it is possible that the severe poxvirus infection predisposed some canaries to productive polyomavirus infection. Poxvirus infection of the thymus and bursa of Fabricius by poxvirus with total loss of architecture in most birds certainly might have compromised both humoral and cell-mediated immunity predisposing the birds to various secondary infections.

Poxvirus is usually transmitted through a break in the skin most commonly caused by vectors such as mosquitoes. But in canaries vectors may not be necessary as the virus can gain entrance through the respiratory route and cause pneumonia. This may result in viraemia with the virus being disseminated throughout the body. Therefore, it is not surprising that poxvirus inclusions were observed in many organs of the canaries in the present study. It is interesting that many mononuclear inflammatory cells, most probably macrophages and most notably in the thymus, bursa, spleen, bone marrow, dermis and lungs, contained intracytoplasmic inclusions of poxvirus, suggesting that these are also target cells in addition to the epithelial cells. Similar "mononuclear cells" containing intracytoplasmic inclusions of poxvirus have been described before in canaries (Giddens *et al*., 1971; Johnson & Castro, 1986). The identity of these "mononuclear cells" has not been investigated and needs further study. Electron microscopy and immunohistochemistry of these mononuclear cells including the cells in the bone marrow and other organs can help determine the identity of theses cells, but no such attempts were made in the present study. The epithelial cells in the bone marrow of the skull that were infected with poxvirus and contained intracytoplasmic inclusions were most probably the epithelial cells of the air sacs that are normally present in the skull.

It was noted that many of the poxvirus inclusions in various organs contained vacuoles within them. It is known that the main components of poxvirus are protein, DNA and lipid (Tripathy & Reed, 2008). It has been reported that that an average poxvirus inclusion body contains 50% extractable lipids (Tripathy & Reed, 2008). Therefore, these lipids get dissolved during the routine processing of tissues for histopathology, leaving vacuoles in the inclusion bodies.

The source of poxvirus in the aviary is most probably the 14 canaries that were added to the aviary without any quarantine. This speculation is supported by the fact that these newly arrived birds were among the first to die in the aviary. This aviary had been in operation for many years and did not have any history of pox in the canaries before. Cleaning and disinfection of the premises, removal of the dead birds and isolation of sick birds and vaccination of rest of the birds for the disease has been successful and there have been no outbreaks for several years.

Acknowledgements

The authors wish to thank Dr R. Crespo, R. Nordhausen, H. Moriyama, R. Gonzales, M. Rezvani, D. Ramirez and L. Alsing for their technical assistance.

References

Bolte, A.L., Meurer, J. & Kaleta, E.F. (1999). Avian host spectrum of avipoxviruses. *Avian Pathology*, *29*, 415–432.

Burnet, F.M. & Bernard, J.E. (1933). A virus disease of the canary of the fowl pox group. *Journal of Pathology and Bacteriology*, *37*, 107–122.

Donnely, T.M. & Crane, L.A. (1983). An epornitic of avian pox in research aviary. *Avian Diseases*, *28*, 517–525.

Garcia, A., Latimer, K.S., Niagro, F.D., Norton, T.M., Campagnoli, R.P., Harmon, B.G., *et al.* (1994). Diagnosis of polyomavirus infections in seedcrackers (*Pyrenestis sp.*) and blue bills (*Spermophaga haematina*) using DNA in situ hybridization. *Avian Pathology*, *23*, 525–537.

Gerlach, H. (1994). Viruses. In B.W. Ritchie (Ed.), *Avian Medicine: Principles and Application* (pp. 862–948). Lake Worth, FL: Wingers Publishing.

Giddens, Jr., W.E., Swango, L.J., Henderson, Jr., J.D., Lewis, R.A., Farner, D.S., Carlos, A., *et al.* (1971). Canary pox in sparrows and canaries (Fringillidae) and in Weavers (Ploceidae). Pathology and host specificity of the virus. *Veterinary Pathology*, *8*, 260–280.

Johnson, B.J. & Castro, A.E. (1986). Canarypox causing high mortality in an aviary. *Journal of the American Veterinary Medical Association*, *189*, 1345–1347.

Kim, T-J. & Tripathy, D.N. (2001). Reticuloendotheliosis virus integration in the fowl poxvirus genome: not a recent event. *Avian Diseases*, *45*, 663–669.

Kim, T-J., Schnitzlein, W.M., McAloose, D., Pessier, A.P. & Tripathy, D.N. (2003). Characterization of an avianpox virus isolated from an andean condor (*Vultur Gryphus*). *Veterinary Microbiology*, *96*, 237–246.

Schnitzlein, W.M., Ghildyal, N. & Tripathy, D.N. (1988). Genomic and antigenic characterization of avipoxviruses. *Virus Research*, *10*, 65–76.

Theil, T., Whiteman, N.K., Tirape, A., Baquero, M.I., Cedeno, V., Walsh, T., *et al.* (2005). Characterization of canarypox-like viruses infecting endemic birds in the Galapagos Islands. *Journal of Wildlife Diseases*, *41*, 342–353.

Tripathy, D.N. & Reed, W.M. (2008). In Y.M. Saif (Ed.), *Diseases of Poultry*, (12th ed., pp. 291–307). Ames, IA: Blackwell Publishing Company.

Wittig, W., Hoffmann K., Müller H. & Johne, R. (2007). Detection of DNA of the finch polyomavirus in diseases of various types of birds in the order Passeriformes. *Berliner und Muenchener Tieraerztliche Wochenschrift*, *120*, 113–119.

Woods, L.W. & Latimer, K.S. (2000). Circovirus Infection of Non-psittacine Birds. *Journal of Avianian Medicine and Surgery*, *14*, 154–163.